MANUAL

RayBio® Glucose Colorimetric Assay Kit

Catalog #: MA-GLU

ISO 13485:2016

Introduction

Glucose, a monosaccharide (or simple sugar), is the most important carbohydrate in biology. It serves as a vital energy source in plants, prokaryotes, and eukaryotes through mechanisms like respiration and fermentation. Glucose levels are tightly regulated in the human body. Failure to maintain blood glucose in the normal range leads to conditions of persistently high (hyperglycemia) or low (hypoglycemia) blood sugar levels. Diabetes mellitus, characterized by sustained hyperglycemia, is a prominent disease associated with impaired blood sugar regulation.

RayBio® Glucose Colorimetric Assay Kit provides a simple, reproducible, and sensitive tool for measuring glucose concentration in plasma, serum, cell lysates, urine and other biological liquid samples. This assay employs a coupled enzymatic reaction system. First, glucose is oxidized by glucose oxidase to form gluconate and hydrogen peroxide (H_2O_2). H_2O_2, in the presence of horseradish peroxidase, then reacts with a colorimetric probe to form a pink-colored product. The optical density measured at 500nm is directly proportional to the glucose concentration in the sample.

Storage

The entire kit may be stored at 2–8 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate (Item A)	A 96-well (12 strips x 8 wells) plate	RT*
Sample Buffer	10 ml	2–8 °C
Glucose Standard	1 vial (100 µl of 100 mg/dL)	2–8 °C
Enzyme Mix Solution	15 ml	2–8 °C

RT = room temperature

**Return unused wells to the pouch containing desiccant pack, reseal along entire edge.*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 500 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. Incubator at 37°C

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. Typically, Glucose levels in human plasma are in the range of 70-110 mg/dL. Plasma samples can be diluted at least 1:5 with Sample Buffer.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. Typically, Glucose levels in human serum are in the range of 70-110 mg/dL. Serum samples can be diluted at least 1:5 with Sample Buffer.

4. Urine samples:

To remove insoluble particles, spin at 10,000 x g for 5 min. Typically, Glucose levels in human urine are in the range of 1-15 mg/dL.

NOTE:
Optimal experimental conditions for samples must be determined by the investigator. A set of serial dilutions is recommended for samples to achieve optimal assay results.

Standard Preparation

To prepare a dilution series of standard in the concentration range of 0 mg/dL – 25 mg/dL (see Table below),

1. Label 8 microtubes #1 through 8 which with the following concentrations: 25, 12.5, 6.25, 3.125, 1.5625, 0.7813, 0.3906, 0 mg/dL.
2. Pipette 150 µL Sample Buffer into labeled tube #1, and 100 µL Sample Buffer into labeled tubes #2 – tube #8.
3. Pipette 50 µL 100 mg/dL Glucose Standard (provided) into tube #1, Mix thoroughly to make 25 mg/dL Glucose Standard.
4. To make the 12.5 mg/dL standard, pipette 100µL tube #1 into the tube labeled #2. Mix thoroughly.
5. Repeat this step with each successive concentration, preparing a dilution series as shown in the Table below. Each time, use 100 µl of the prior concentration until the 0.3906 mg/dL is reached. Mix each tube thoroughly before the next transfer. Standards should be prepared fresh, mixed thoroughly, and used immediately.

Labeled Tubes	Glucose Standard (µL)	Sample Buffer (µL)	Standard Conc. (mg/dL)
1	50 µL of 100mg/dL Stock	150µL	25
2	100 µL of Tube #1	100 µL	12.5
3	100 µL of Tube #2	100 µL	6.25
4	100 µL of Tube #3	100 µL	3.125
5	100 µL of Tube #4	100 µL	1.5625
6	100 µL of Tube #5	100 µL	0.7813
7	100 µL of Tube #6	100 µL	0.3906
8	0µL	100 µL	0

Assay Procedure

Each Glucose standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Pre-warm the Enzyme Mix Solution at 37°C (in the dark) for at least five minutes.
2. Add 30 µL of the diluted Glucose standards or samples to the 96-well Microplate.
3. Initiate the reaction by adding 150 µL Enzyme Mix Solution to each well (pre-heat reagent at 37°C).
4. Cover with the plate cover. Carefully shake the plate for a few seconds to mix.
5. Incubate the plate for 10 minutes at 37°C in the dark.
6. Measure the absorbance at 500nm using a plate reader.

Calculation of Results

Subtract the blanks

Average the absorbance value of the blank wells (Standard 0 mg/dL) and subtract this from the absorbance values of all the other wells. These are the corrected absorbance.

Plotting the standard curves

Make a plot of corrected absorbance at 500nm as a function of Glucose concentration.

Determination of sample Glucose concentration

Glucose (mg/dL) = $\frac{OD_{Sample} - OD_{Blank}}{Slope} \times DF$

OD_{Sample} = Optical density (OD) reading of the Sample

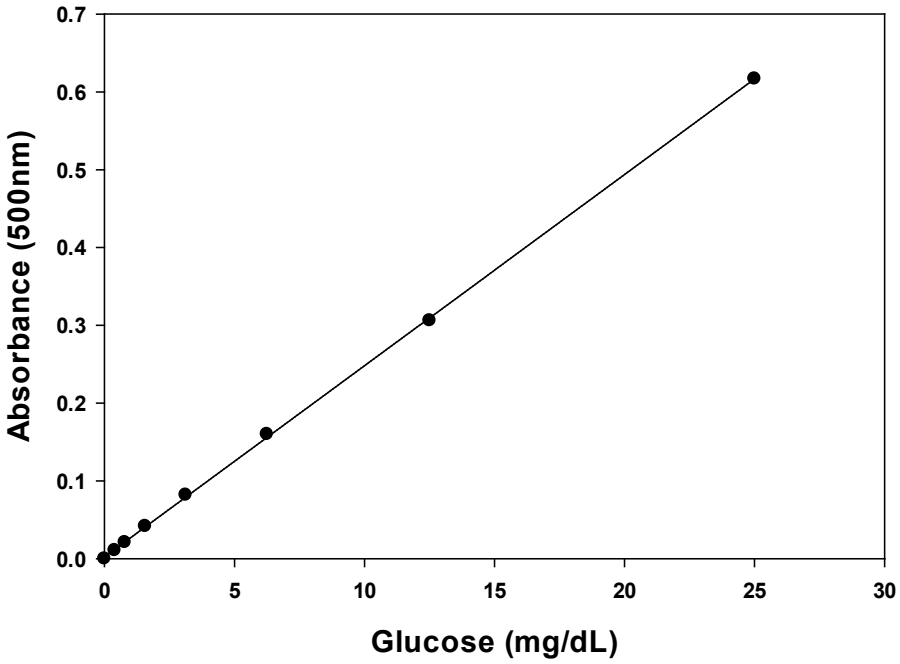
OD_{Blank} = Optical density (OD) reading of the Blank (Standard 0 mg/dL)

Slope is from the plot of Glucose concentration vs. Absorbance shown in Typical data below

DF = Sample Dilution factor (DF = 1 for undiluted samples)

Note: If the calculated Glucose concentration of the sample is higher than 25 mg/dL, dilute the sample in Sample Buffer and repeat the assay.

A. Typical Data



These standard curves are for demonstration only. A standard curve must be run with each assay.

B. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 1.7%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 3.6%.

This product is for research use only.